

# ALFALFA

## Population Density and Harvest Maturity Effects on Leaf and Stem Yield in Alfalfa

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### ABSTRACT

A system has been proposed using alfalfa (*Medicago sativa* L.) as a biofuel feedstock, where the stems would be processed to produce energy and the leaves used as a livestock feed. Our objectives were to evaluate the effects and interactions of environment, population density, and harvest maturity on leaf and stem yield of alfalfa germplasms differing in fall dormancy and leaf/stem ratio. Four alfalfa germplasms established at four population densities (450, 180, 50, and 16 plants  $m^{-2}$ ) were harvested at the early bud and green pod maturity stages and evaluated in three environments for leaf and stem yield. All main effects and several two-way interactions influenced leaf and stem yield ( $P > 0.01$ ). The population density  $\times$  harvest maturity interaction had the greatest impact on yield. Leaf and stem yield per unit area increased as population density increased from 16 to 450 plants  $m^{-2}$  at the early bud stage. In contrast, leaf and stem yield increased as population density increased from 16 to 180 plants  $m^{-2}$ , but decreased dramatically at 450 plants  $m^{-2}$  at the green pod maturity stage. Delaying harvest until the green pod maturity stage and decreasing population density to 180 plants  $m^{-2}$  maximized both leaf and stem yield in all four alfalfa germplasms studied. Decreasing population density to 180 plants  $m^{-2}$ , and harvesting twice per season at a later maturity stage would be an effective management strategy for maximizing yield in an alfalfa biomass energy or biofuel production system.

ALFALFA LEAF and stem proportions influence its value as a livestock feed and as a biomass energy crop. For livestock feeding, harvest at bud to early flower is recommended to provide forage with high to medium nutrient concentration. Sheaffer et al. (2000) reported decreased crude protein (CP) and increased fiber content as well as changes in leaf/stem ratio in alfalfa forage harvested at advancing maturity stages. Alfalfa harvested at midbud had greater leaf yield than stem yield, while at early flower, leaf and stem yields were nearly the same. At late flower, the stem portion of the forage outyielded the leaves (Sheaffer et al., 2000). Other researchers also have reported decreases in leaf concentration with advancing maturity (Fick and Holthausen, 1975; Kilcher and Heinrichs, 1974).

In a biomass energy production system, alfalfa forage would be fractionated into stems and leaves. The stems would be processed to generate energy or a biofuel, and

the leaves would be sold as a high-protein livestock feed supplement (Delong et al., 1995). A biomass energy production system adds value to the stem component of alfalfa forage and may favor a shift to harvesting at more mature stages to increase stem yield. The advantage of using alfalfa for biomass energy production compared with other crops is having a secondary income from selling the leaves as a high value feed supplement. To reach the economic potential of an alfalfa biomass energy production system, both leaf and stem yield will need to be maximized.

Marquez-Ortiz et al. (1999) reported that individual stem diameter was heritable and controlled by additive genetic effects and suggested that selection for larger stems in alfalfa was feasible. Volenec et al. (1987) found that selection for high yield per stem was an effective method to increase forage yield, but plants may have less digestible, larger stems. Germplasms from southern Europe, referred to as *Flemish* types, are a genetic source for large stem size and resistance to foliar diseases, but display early maturity, lack winterhardiness, and are susceptible to root and crown diseases (Barnes et al., 1977).

Commercial alfalfa breeding programs have developed cultivars with high forage quality for feeding dairy livestock. Programs have attempted to enhance forage quality by directly manipulating forage-quality components such as protein, fiber, or lignin content (Huset et al., 1991; Kephart et al., 1990) and by increasing the leaf portion of the forage. Approaches to increasing leaf concentration in alfalfa have included increased leaflet size (Leavitt et al., 1979) and higher leaflet number (Bingham and Murphy, 1965; Ferguson and Murphy, 1973; Brick et al., 1976). Multifoliolate alfalfa types produce four or more leaflets per leaf compared with three leaflets for the normal trifoliolate alfalfa leaf. Several studies have reported greater leaf/stem ratios in alfalfa cultivars with the multifoliolate trait compared with the normal trifoliolate cultivars (Ferguson and Murphy, 1973; Brick et al., 1976; Volenec and Cherney, 1990; Juan et al., 1993). Results of these studies show potential for improving leaf concentration in alfalfa.

Several studies have documented alfalfa population density effects on stem, leaf, and total forage yield. No association between alfalfa population density and production year total forage yield was demonstrated by Kephart et al. (1992) or Sund and Barrington (1976). Other studies reported increased production year forage yield as alfalfa population densities increased (Bolger and Meyer, 1983; Hansen and Krueger, 1973; Volenec et al., 1987; Cowett and Sprague, 1962; Rumbaugh, 1963). Several researchers stated that the yield of individual

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alfalfa stems and number of stems per plant decreased as population density increased (Bolger and Meyer, 1983; Kephart et al., 1992; Volenec et al., 1987; Cowett and Sprague, 1962; Rumbaugh, 1963). Hansen and Krueger (1973) reported that higher population densities produced finer stems, decreased root and crown weights, and increased leaf drop due to shading. Volenec et al. (1987) stated that stem diameter and nodes per stem decreased as population density increased and that shoot weight was an important component of plant weight, especially at high population densities. Rumbaugh (1963) reported a differential response to increased population densities in the two varieties they studied. At 3.5 plants  $m^{-2}$  individual plants of 'Teton' outyielded plants of 'Ranger', but from 14 to 133 plants  $m^{-2}$  plants of both cultivars yielded the same.

A two-cut harvest regime taken at late flower to early pod has been proposed for an alfalfa biomass energy production system to maximize stem yield, enhance wildlife habitat, and minimize harvest costs (Sheaffer et al., 2000). Variation for leaf and/or stem yield is evident in different genetic sources of alfalfa (Barnes et al., 1977; Sheaffer et al., 2000). Both plant maturity and population density affect the expression of leaf and stem proportion or concentration in alfalfa (Fick and Holthausen, 1975; Sheaffer et al., 2000; Bolger and Meyer, 1983; Kephart et al., 1992; Volenec et al., 1987; Hansen and Krueger, 1973; Rumbaugh, 1963). The key to success in an alfalfa biomass energy production system would be to develop management systems and germplasms that would maximize both leaf and stem yield. Our objectives were to evaluate the effects and interactions of environment, population density, and harvest maturity on leaf and stem yield of alfalfa germplasms differing in fall dormancy and leaf/stem ratio.

## MATERIALS AND METHODS

### Plant Materials

Four alfalfa germplasms differing in fall dormancy and leaf/stem ratio were chosen for this study. 'MP2000' is a commercial multileaf alfalfa cultivar theoretically selected for greater leaf/stem ratio. MWNC-4 (UMN 3041) is an experimental population selected for resistance to *Phytophthora* (*Phytophthora medicaginis* Hansen and Maxwell) and *Aphanomyces* (*Aphanomyces euteiches* Drechs.) root rots and root-lesion nematode (*Pratylenchus penetrans* Cobb, Filipjev and Schur-Stekhoven), and is adapted to the upper Midwest region. These two germplasms are moderately dormant with fall dormancy ratings between 2 and 3. 'New Europa' is a southern European alfalfa cultivar of Flemish origin (Barnes et al., 1977). ORCA-WTS (UMN 3040) is an experimental population selected from a different Flemish cultivar for large, nonlodging, woody stems at the late-flower maturity stage. Both of these Flemish germplasms were less dormant, and have dormancy ratings of 5.

### Experimental Design

The experimental design was a randomized complete block with four replicates in a split plot factorial arrangement of the subplot treatments, where four population densities were the whole plots, and all combinations of the four germplasms and two plant maturities were the subplots.

The four population densities chosen for this study were 16, 50, 180, and 450 plants  $m^{-2}$ . The 16 plants  $m^{-2}$  population density treatment was chosen to represent plant spacings used in a plant breeder's nursery allowing ample space between plants to evaluate alfalfa populations on an individual plant basis. The 450 plants  $m^{-2}$  treatment was chosen to represent the drilled seeding rate growers typically use to establish alfalfa. The 180 and 50 plants  $m^{-2}$  populations density treatments were chosen as intermediates between the drilled seeding rate and spaced plant populations densities used by plant breeders.

The 16 plants  $m^{-2}$  population density plots were  $1.2 \times 3.0$  m and consisted of 50 plants spaced 30 cm apart. The 24 interior plants were harvested for forage yield. The 50 plants  $m^{-2}$  treatment was established in 0.9 by 0.9 m plots and consisted of 49 plants spaced at 15-cm spacings. The 180 plants  $m^{-2}$  had 7.5 cm between plants in 0.45 by 0.45 m plots. For both the 50 and 180 plants  $m^{-2}$  densities, the interior 25 plants were harvested for forage yield. These first three population density treatments were seeded by hand with two to three seeds per hole and thinned to one plant per hole 15 to 20 d after seeding. The 450 plants  $m^{-2}$  population density treatment was mechanically seeded using a Wintersteiger Plotman plot planter (Wintersteiger, Salt Lake City, UT), at a rate of 11 kg  $ha^{-1}$  in 1.8 by 2.0 m plots with 10 rows drilled 12 cm apart at an approximate population density of 450 plants  $m^{-2}$ . In these plots a 0.30 by 0.45 m area was harvested for forage yield. Alfalfa plant population densities can change with time, but we had minimal loss of plants in the three spaced plant density treatments. A border plant or two was lost over time, but no losses occurred among the plants we harvested to estimate yield. We likely had some thinning of the stand in the solid seeded plots, but there were no gaps in the rows in the sections of the plots we harvested to estimate yield. No obvious losses were evident because alfalfa plants in the solid seeded stand increased in size as smaller plants were lost. Therefore, we feel we had negligible change in plant population densities over the time span of this study.

The experiment was planted at the Sand Plains Research Farm, Becker, MN (Hubbard loamy sand; sandy, mixed, Udorthentic Haploboralls) on 20 Aug. 1996 and at the Minnesota Agricultural Experiment Station at Rosemount, MN (Tallula silt loam; coarse silty, mixed, mesic Typic Hapludolls) on 19 May 1997. Soil pH, P, and K levels were adjusted to levels recommended for alfalfa production (Rhem and Schmitt, 1989). Weeds were controlled by hand weeding. All plots were sprayed periodically with Pounce 25 WP (a.i. Permethrin (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate) to control potato leafhopper [*Empoasca fabae* (Harris)]. Plots were harvested at two maturity stages: (i) early bud when 10 to 33% of the stems in the plot had flower buds; and (ii) green pod when >10% of the stems had green seedpods but <10% of the stems had mature brown pods. Because population density influences the rate at which alfalfa matures, all plots were harvested when the plants in the 50 plants  $m^{-2}$  plots had reached the target maturity. Plots were hand-harvested at a stubble height of 5 cm at the early bud stage of maturity either three or four times per season on 12 June, 7 July, and 6 Aug. 1997 and on 28 May, 1 and 23 July, and 21 Aug. 1998 at Becker, MN. At the Rosemount site, harvest dates were on 21 May, 26 June, 22 July, and 25 Aug. 1998. Plots were harvested at the green pod stage of maturity twice per season on 30 June and 8 Aug. 1997, and on 30 June and 4 Aug. 1998 at Becker, MN, and on 23 June and 10 Aug. 1998 at Rosemount, MN. In the analysis of variance, we considered each harvest year at a location as an environment. Fresh forage subsamples were weighed, dried

at 55°C in forced-air ovens, and weighed again to estimate dry matter yield.

To assess leaf and stem yield at all population densities and maturities, each plot was subsampled by randomly selecting 25 stems at the target maturity. These subsamples were dried at 55°C in forced-air ovens, weighed, and then stems were separated from the leaves (all floral components remained with the leaf portion). The leaf and stem portions were reweighed and leaf and stem concentrations were estimated for each plot. Leaf and stem yield per plot were calculated by multiplying the leaf or stem concentrations of the subplot by the total yield for each plot. All yields are reported on a grams of dry matter per square meter basis.

Analysis of variance was conducted to determine the effects of environment, plant population density, plant maturity, and alfalfa germplasms on total leaf, stem, and forage yield for the season (PROC GLM, SAS Inst., 1998). Environments were considered random and plant population density treatments, harvest maturities, and alfalfa germplasms were considered fixed. Least square means for leaf, stem, and forage yield for the main effects and interactions of the three environments, two harvest maturities, four plant population densities, and four alfalfa germplasms were compared with the PDIF option of PROC GLM (SAS Inst., 1998). Leaf yield and concentration means at each harvest for all population density  $\times$  maturity stage treatment combinations within each environment were also compared using the least significant difference (Steel and Torrie, 1980). Significance was declared at  $P < 0.05$  unless otherwise indicated.

## RESULTS AND DISCUSSION

Environment, population density, maturity at harvest, and germplasms impacted leaf, stem, and total forage yield (Table 1). Several two-way interactions among these main effects accounted for considerable variation for all yield traits.

### Environment

Total forage yield at Becker in 1997 was  $847 \pm 28$  g  $m^{-2}$ , while in 1998 total forage yields were  $1206 \pm 25$

and  $1228 \pm 23$  g  $m^{-2}$  for Becker and Rosemount, respectively. Winter injury occurred in the spring of 1997 at Becker, but no winter injury was evident in the spring of 1998 at either Becker or Rosemount. No plants were lost from this injury, but losses in yield contributed to environment  $\times$  population density, environment  $\times$  germplasm, and environment  $\times$  maturity interactions.

Winter injury at Becker in 1997 was more severe and caused greater yield loss in the 16 plants  $m^{-2}$  population density treatment compared with the other three density treatments for both leaf and stem (Fig. 1A and B). Leaf yield responded similarly to population density in all three environments with the greatest yield at 180 plants  $m^{-2}$ , followed by 450 plants  $m^{-2}$  yielding slightly more or the same as 50 plants  $m^{-2}$ , with the lowest leaf yield at 16 plants  $m^{-2}$  (Fig. 1A). The population density treatments ranked differently in the three environments for stem yield (Fig. 1B). Stem yield was slightly greater at 180 plants  $m^{-2}$  compared with 450 plants  $m^{-2}$ , but not different from 50 plants  $m^{-2}$ , with the 16 plants  $m^{-2}$  having the lowest stem yield at Becker in 1997. In 1998 at Becker, stem yield was greatest at the 180 plants  $m^{-2}$  treatment, followed by equivalent yields at both 450 and 50 plants  $m^{-2}$ , and the 16 plants  $m^{-2}$  had the lowest yield. At Rosemount in 1998, the 450 and 180 plants  $m^{-2}$  treatments yielded the same followed by the 50 plants  $m^{-2}$ , and the 16 plants  $m^{-2}$  had the lowest stem yield.

New Europa and ORCA-WTS are less winterhardy than MP2000 and MWNC-4 and sustained a greater winter injury at the first early bud harvest at Becker in 1997 (data not shown). This injury led to lower leaf yield for both New Europa and ORCA-WTS compared with MP2000 and MWNC-4 at Becker in 1997 (Fig. 2A). No differences in leaf yield were found among the germplasms for either location in 1998. The winter injury effect on stem yield at Becker in 1997 varied among the winterhardy and nonwinterhardy germplasms (Fig. 2B). Stem yield was not different between MP2000 and

**Table 1.** Seasonal leaf, stem, and total forage yield mean squares from the analysis of variance over three environments, four populations densities, two harvest maturity stages, and four alfalfa germplasms.

Source	df	Mean squares		
		Leaf	Stem	Total forage
Environment (E)	2	545 108***	3 245 500***	6 451 987***
Replication (R)[E]	9	19 813	26 735	80 338
Population density (D)	3	1 004 872**	981 983*	3 958 947**
E $\times$ D	6	62 531*	113 672*	318 668*
R $\times$ D [E]	27	19 711	34 901	104 662
Germplasm (G)	3	49 894**	89 316***	137 206*
Maturity stage (M)	1	134 782***	2 860 916***	1 760 797***
G $\times$ M	3	7 189	11 625	24 855
D $\times$ G	9	30 833**	30 368*	119 784**
D $\times$ M	3	225 462***	242 878***	917 099***
E $\times$ G	6	38 428**	41 752**	160 686**
E $\times$ M	2	254 775***	17 159	358 357***
D $\times$ G $\times$ M	9	15 959	20 427	64 924
E $\times$ G $\times$ M	6	11 147	8 518	34 770
E $\times$ D $\times$ G	18	11 286	9 498	36 248
E $\times$ D $\times$ M	6	4 268	7 994	20 008
E $\times$ D $\times$ G $\times$ M	18	9 184	5 438	24 623
Error b	252	11 249	13 999	45 101
CV		21	20	19

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

\*\*\* Significant at the 0.001 level.



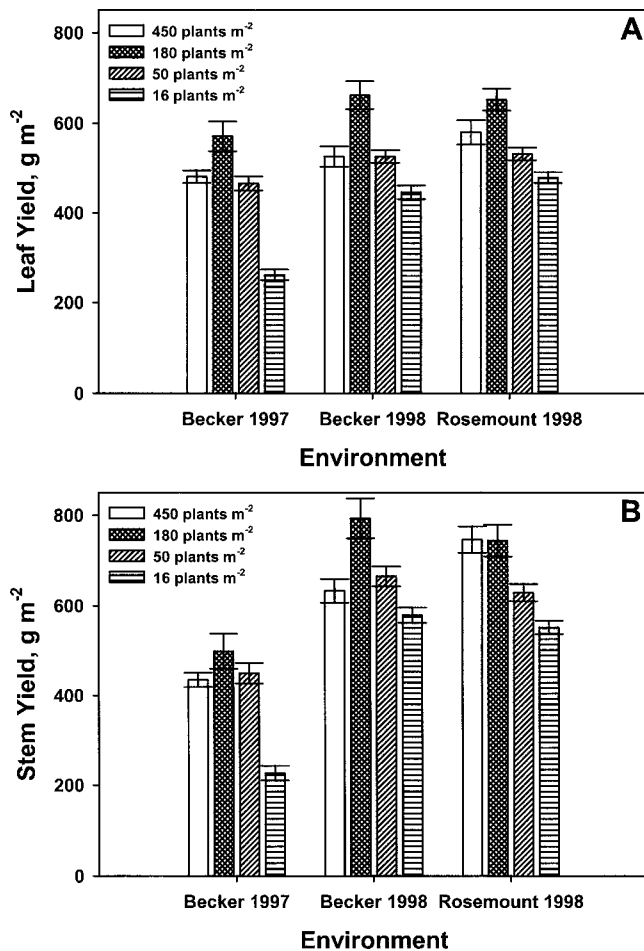


Fig. 1. Means ( $\pm 1$  SE) for (A) leaf and (B) stem yield for each plant population density treatment in each environment.

MWNC-4 or between New Europa and ORCA-WTS, and New Europa and ORCA-WTS yielded the same as MP2000, but less than MWNC-4. At both locations in 1998, ORCA-WTS had greater stem yield than MP2000.

Total forage and leaf yield at the early bud stage was different from the green pod stage among the environments evaluated in our study. An environment  $\times$  maturity stage interaction occurred because total forage yield was much greater at green pod than early bud at Becker in 1997, slightly greater at green pod than early bud at Becker in 1998, and there was no difference between maturity stages at Rosemount in 1998 (Fig. 3A). Leaf yield at early bud was less than green pod at Becker in 1997, while early bud was greater than green pod at both locations in 1998 (Fig. 3B). The greater differences in yield between the maturity stages at Becker in 1997 was biased by winter injury at the first early bud harvest in late spring. Plots harvested at green pod in late June in 1997 had extra time to compensate and recover from the winter injury. Stem yield was greater at green pod than early bud in all three environments (data not shown).

### Population Density and Maturity Stage

Population density  $\times$  maturity interactions had a large impact on leaf, stem, and forage yield (Table 1).

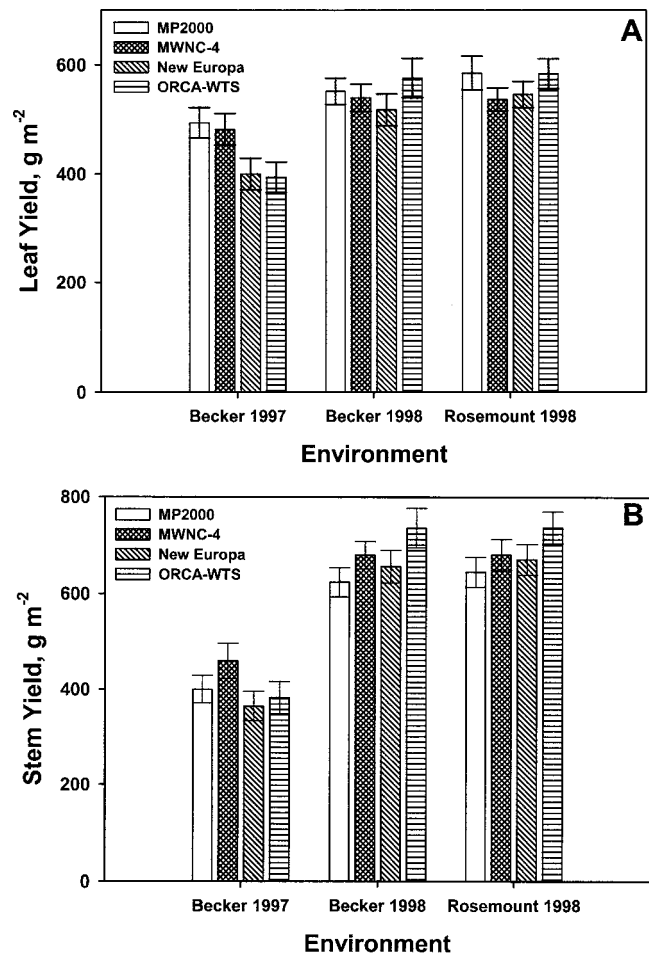


Fig. 2. Means ( $\pm 1$  SE) for (A) leaf and (B) stem yield for each germplasm in each environment.

As population density increased from 16 to 450 plants  $m^{-2}$  at the early bud stage, leaf, stem, and total forage yield per unit area also increased (Fig. 4A, B, and C), agreeing with previous reports (Cowett and Sprague, 1962; Bolger and Meyer, 1983; Volenec et al., 1987). In contrast, at the green pod maturity stage, leaf, stem, and total forage yield increased as population density increased from 16 to 180 plants  $m^{-2}$ , but at 450 plants  $m^{-2}$  all yields decreased dramatically (Fig. 4A, B, and C). This decrease in yield at 450 plants  $m^{-2}$  was possibly due to plant competition for water, nutrients, and light at this denser plant population, producing finer stems, decreased root and crown weights, and increased leaf drop due to shading (Hansen and Krueger, 1973). Delaying harvest until the green pod maturity stage presumably increased the incidence of foliar diseases that also could have contributed to yield reduction, especially in the greater population density treatment (Undersander et al., 2000).

At the two lower plant density treatments (16 and 50 plants  $m^{-2}$ ) as well as the 450 plants  $m^{-2}$  density treatment, leaf yield was greater at early bud, while stem yield was greater for green pod at all population densities (Fig. 4A and B), agreeing with previous reports (Fick and Holthausen, 1975; Juan et al., 1993; Kilcher and Heinrichs, 1974; and Sheaffer et al., 2000). Total

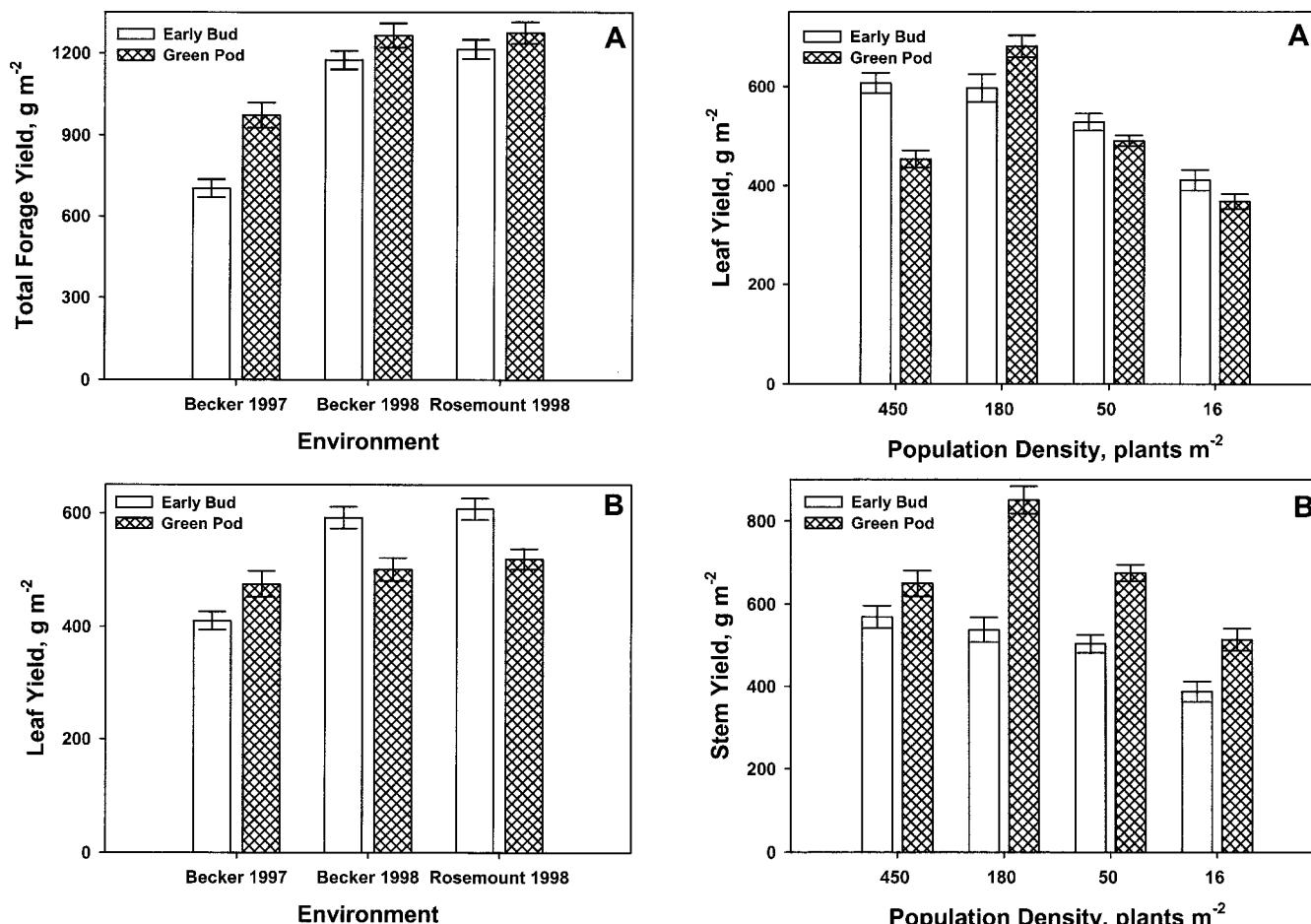


Fig. 3. Means ( $\pm 1$  SE) for (A) season total forage and (B) leaf yield for each harvest maturity stage in each environment.

seasonal forage yield was the same for both maturity stages at 450 plant  $m^{-2}$  (Fig. 4C). The decline in leaf yield at the later harvest maturity stage was offset by a gain in stem yield, agreeing with results reported by Sheaffer et al. (2000). Seasonal total forage yield was the same at both the 50 and 450 plants  $m^{-2}$  treatments for both harvest maturity stages (Fig. 4C). It is possible that total forage yield was limited by competition at the 450 plant  $m^{-2}$  treatment, and insufficient plant population per unit area at the 50 plants  $m^{-2}$  treatment.

At the 180 plants  $m^{-2}$  population density treatment, leaf yields harvested at the two maturities stages were reversed compared with the other three population density treatments (Fig. 4A). All three yield components at 180 plants  $m^{-2}$  were greater when harvested at the green pod maturity stage compared with the early bud stage (Fig. 4A, B, and C). Leaf, stem, and season total forage yield were maximized in our study at this intermediate population density treatment by delaying harvest until the green pod maturity stage. A lower but adequate plant population per unit area may have decreased plant competition, shading, and incidence of disease, reducing leaf loss and increasing stem growth at the later harvest maturity stage.

### Germplasms

Germplasms ranked differently for leaf and stem yield at the different population densities, causing a germ-

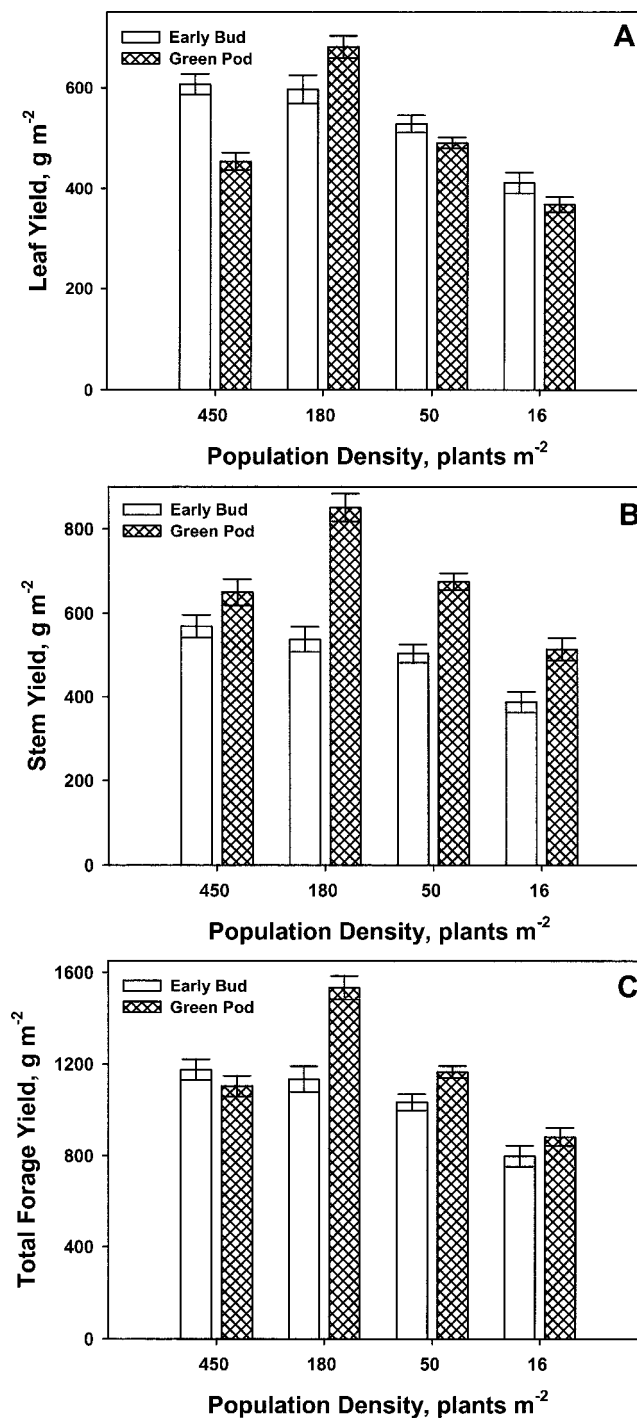


Fig. 4. Means ( $\pm 1$  SE) for (A) leaf, (B) stem, and (C) total forage yield for each population density treatment  $\times$  harvest maturity stage combination.

plasm  $\times$  population density interaction (Fig. 5A and B). Mean leaf yields among the four germplasms were the same within each of the 450 and 180 plants  $m^{-2}$  population density treatments (Fig. 5A). Leaf yield was greater at 180 compared with 450 plants  $m^{-2}$  for MP2000, MWNC-4, and ORCA-WTS, but for New Europa these two population density treatments yielded the same. Our results differ from Sheaffer et al. (2000), who reported differences in leaf yield among alfalfa varieties seeded at 11 kg  $ha^{-1}$  (450 plants  $m^{-2}$ ). At 50

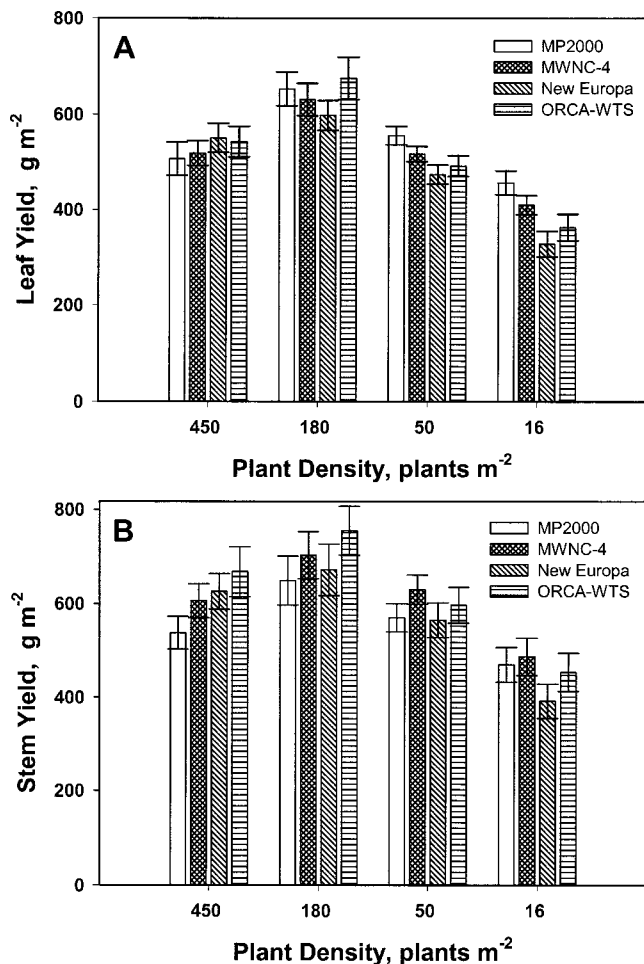


Fig. 5. Means ( $\pm 1$  SE) for (A) leaf and (B) stem yield for each germplasm  $\times$  population density treatment combination.

plants  $m^{-2}$ , MP2000 had greater leaf yield compared with the other three germplasms. MWNC-4 had greater leaf yield than New Europa, but yielded the same as ORCA-WTS, and no difference in leaf yield between New Europa and ORCA-WTS was shown. At 16 plants

$m^{-2}$ , MP2000 and MWNC-4 were similar, but both were greater in leaf yield than New Europa. MWNC-4 yielded the same as ORCA-WTS, while no difference in leaf yield was shown between ORCA-WTS and New Europa. Results demonstrate differences and shifts in rank order among germplasms for leaf yield only in the more open, less dense plant population treatments.

Stem yield was comparable among the four germplasms within each of the 180 and 50 plants  $m^{-2}$  population density treatments. New Europa and ORCA-WTS had greater stem yield at 180 compared with 50 plants  $m^{-2}$ , but these two populations density treatments had similar stem yield for both MP2000 and MWNC-4. At 16 plants  $m^{-2}$ , MP2000, MWNC-4, and ORCA-WTS were equivalent for stem yield, while New Europa yielded less than MP2000 and MWNC-4, but the same as ORCA-WTS. At 450 plants  $m^{-2}$ , MWNC-4, New Europa, and ORCA-WTS had comparable stem yield. MP2000 had less stem yield compared with the two Flemish germplasms (New Europa and ORCA-WTS), but yielded the same as MWNC-4. Our results differ from Sheaffer et al. (2000), who reported no differences for stem yield among alfalfa varieties established at a seeding rate of 450 plants  $m^{-2}$ .

We had postulated that MP2000, a multifoliolate cultivar, might have greater leaf yield compared with the other trifoliolate germplasms. MP2000 demonstrated some differences in leaf yield at the different population density treatments, but no consistent response was evident. When plants were spaced 15 to 30 cm apart (50–16 plants  $m^{-2}$ ), there was a trend toward greater leaf yield for MP2000. However, at more dense plant populations traditionally used to produce alfalfa, MP2000 had the same leaf yield as the other trifoliolate germplasms evaluated in our study.

### Leaf Yield and Concentration

At all six green pod maturity stage harvests, the 180 plants  $m^{-2}$  density treatment had the greatest leaf yield (Table 2). Leaf yield response to population density

Table 2. Leaf yield and concentration by harvest for three environments for all population density  $\times$  maturity stage combinations.

	Early bud maturity stage								Green pod maturity stage			
	Harvest 1		Harvest 2		Harvest 3		Harvest 4		Harvest 1		Harvest 2	
	$g\ m^{-2}$	$g\ kg^{-1}$	$g\ m^{-2}$	$g\ kg^{-1}$	$g\ m^{-2}$	$g\ kg^{-1}$	$g\ m^{-2}$	$g\ kg^{-1}$	$g\ m^{-2}$	$g\ kg^{-1}$	$g\ m^{-2}$	$g\ kg^{-1}$
<b>Becker 1997</b>												
450 plants $m^{-2}$	260	535	117	589	135	592	—	—	190	417	262	554
180 plants $m^{-2}$	172	541	140	584	155	646	—	—	288	452	384	565
50 plants $m^{-2}$	203	518	108	610	114	603	—	—	250	420	259	540
16 plants $m^{-2}$	84	531	62	610	89	689	—	—	133	433	135	548
LSD <sub>0.05</sub>	45	NS†	27	NS	32	NS	—	—	80	NS	54	NS
<b>Becker 1998</b>												
450 plants $m^{-2}$	246	520	181	502	97	674	90	444	232	353	193	440
180 plants $m^{-2}$	308	503	149	510	142	539	78	439	394	388	311	451
50 plants $m^{-2}$	258	506	148	466	95	472	87	576	274	364	208	426
16 plants $m^{-2}$	216	467	128	463	86	554	59	552	219	354	172	420
LSD <sub>0.05</sub>	27	23	NS	27	NS	89	NS	NS	74	NS	53	NS
<b>Rosemount 1998</b>												
450 plants $m^{-2}$	234	436	167	507	161	566	132	469	267	349	218	414
180 plants $m^{-2}$	285	475	137	545	133	561	93	492	332	377	334	483
50 plants $m^{-2}$	237	441	132	518	108	552	95	582	249	384	233	448
16 plants $m^{-2}$	210	464	115	507	98	551	86	572	244	392	200	444
LSD <sub>0.05</sub>	47	NS	NS	NS	28	NS	NS	NS	61	29	62	29
Harvest dates	21 May–12 June		26 June–8 July		22 July–6 Aug.		21–25 Aug.		23–30 June		4–10 Aug.	

† Not significant ( $P > 0.05$ ).



treatments was variable at the early bud maturity stage harvests. Changes in population density impacted leaf concentration only for the first three early bud harvests at Becker in 1998 and for the green pod harvests at Rosemount in 1998. For the first two early bud harvests at Becker in 1998, leaf concentration was lower at the lower population densities. For the third early bud harvest, leaf yields were the same, but leaf concentration was higher for the 450 plants  $m^{-2}$  density. At the green pod harvests at Rosemount in 1998, leaf concentration was lower at the 450 plants  $m^{-2}$  compared with the other population density treatments. The lower leaf concentration with advancing maturity, especially at the first green pod harvest, agreed with previous reports by Fick and Holthausen (1975), Kilcher and Heinrichs (1974), and Sheaffer et al. (2000). Even though leaf concentration declined at the later maturity stage, the reduction in plant population to 180 from 450 plants  $m^{-2}$  increased leaf yield per unit area at green pod (Table 2). Less competition for nutrients, water, and light and increased airflow decreasing incidence of foliar disease could be possible explanations for the greater leaf yield at this lower population density treatment. Delaying harvest until green pod allows development of larger stems with more nodes (Volenc et al., 1987), which may result in increased leaf production. It is likely that the combination of both of these factors increased in leaf yield at the green pod and 180 plants  $m^{-2}$  treatment combination.

## CONCLUSIONS

Historically, recommended management systems have emphasized harvesting alfalfa forage at immature growth stages to maximize the leaf component and the nutrient value to ruminant livestock. A biomass energy production system gives value to the stem component of the alfalfa forage, making it as important as leaf yield for the economics of this production system. Environment, population density, maturity stage at harvest, and germplasm source influenced all yield components in our study. The interaction between plant population density and maturity stage at harvest had the greatest impact on leaf and stem yield. The overall greatest leaf, stem, and total forage yield occurred when plant density was 180 plants  $m^{-2}$  and harvests were timed at the green pod maturity stage for all four germplasms. A two-cut management system taken at green pod at a population density of 180 plants  $m^{-2}$  produced more total forage than a three- or four-cut management system harvested at early bud at any population density. We propose that decreasing stand density to 180 plant  $m^{-2}$  and delaying harvest until green pod and harvesting twice per season would maximize both leaf and stem yield for an alfalfa biomass energy production system.

## REFERENCES

- Barnes, D.K., E.T. Bingham, R.P. Murphy, O.J. Hunt, D.F. Beard, W.H. Skrdla, and L.R. Teuber. 1977. Alfalfa germplasm in the United States: Genetic vulnerability, use, improvement, and maintenance. USDA Tech. Bull. 1571. USDA, Washington, DC.
- Bingham, E.T., and R.P. Murphy. 1965. Breeding and morphological studies on multifoliolate selection of alfalfa, *Medicago sativa* L. Crop Sci. 5:233–235.
- Bolger, T.P., and D.W. Meyer. 1983. Influence of plant density on alfalfa yield and quality. p. 37–41. In Proc. Am. Forage and Grassl. Conf., Eau Claire, WI. 23–26 January. American Forage and Grassland Council. Lexington, KY.
- Brick, M.A., A.K. Dobrenz, and M.H. Schonhorst. 1976. Transmittance of the multifoliolate leaf characteristic in non-dormant alfalfa. Agron. J. 68:134–136.
- Cowett, E.R., and M.A. Sprague. 1962. Factors affecting tillering in alfalfa. Agron. J. 54:294–297.
- Delong, M.M., D.R. Swanberg, E.A. Oelke, C. Hanson, M. Onischak, M.R. Schmid, and B.C. Wiant. 1995. Sustainable biomass energy production and rural economic development using alfalfa as a feedstock. p.1582–1591. In D.L. Klass (ed.). Second Biomass Conference of the Americas: Energy, Environment, Agriculture, and Industry, 21–24 August 1995; Portland OR. National Renewable Energy Laboratory, Golden CO.
- Ferguson, J.E., and R.P. Murphy. 1973. Comparison of trifoliolate and multifoliolate phenotypes of alfalfa (*Medicago sativa* L.). Crop Sci. 13:463–465.
- Fick, G.W., and R.S. Holthausen. 1975. Significance of parts other than blade and stems in leaf-stem separations of alfalfa herbage. Crop Sci. 15:259–262.
- Hansen, L.H., and C.R. Krueger. 1973. Effect of establishment method, variety, and seeding rate on the production and quality of alfalfa under dryland and irrigation. Agron. J. 65:755–759.
- Huset, D.E., D.A. Schnebbe, J.L. Kugler, and M.A. Peterson. 1991. Registration of WL322HQ alfalfa. Crop Sci. 31:1699–1700.
- Juan, N.A., C.C. Sheaffer, D.K. Barnes, D.R. Swanson, and J.H. Halgerson. 1993. Leaf and stem traits and herbage quality of multifoliolate alfalfa. Agron. J. 85:1121–1127.
- Kephart, K.D., D.R. Buxton, and R.R. Hill, Jr. 1990. Digestibility and cell wall components of alfalfa following selection for divergent herbage lignin concentration. Crop Sci. 30:207–212.
- Kephart, K.D., E.K. Twidwell, R. Bortnem, and A. Boe. 1992. Alfalfa yield component responses to seeding rate several years after establishment. Agron. J. 84:827–831.
- Kilcher, M.R., and D.H. Heinrichs. 1974. Contributions of stems and leaves to the yield and nutrient level of irrigated alfalfa at different stages of development. Can. J. Plant Sci. 54:739–742.
- Leavitt, J.R.C., A.K. Dobrenz, and J.E. Stone. 1979. Physiological and morphological characteristics of large and small leaflets in alfalfa genotypes. Agron. J. 71:529–532.
- Marquez-Ortiz, J.J., J.F.S. Lamb, L.D. Johnson, D.K. Barnes, and R.E. Stucker. 1999. Heritability of crown traits in alfalfa. Crop Sci. 39:38–43.
- Rhem, G., and M.A. Schmitt. 1989. Fertilizing alfalfa in Minnesota. AG-FO-3814. Minnesota Extension Service. University of Minnesota, St. Paul, MN.
- Rumbaugh, M.D. 1963. Effects of population density on some components of yield of alfalfa. Crop Sci. 3:423–424.
- SAS Institute, Inc. 1998. Version 7.0 ed. SAS Institute, Inc. Cary, NC.
- Sheaffer, C.C., N.P. Martin, J.F.S. Lamb, G.R. Cuomo, J.G. Jewett, and S.R. Quering. 2000. Leaf and stem properties of alfalfa entries. Agron. J. 92:733–739.
- Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. 2nd ed. McGraw-Hill, New York.
- Sund, J.M., and G.P. Barrington. 1976. Alfalfa seeding rates: Their influence on dry matter yield, stand density, and survival, root size and forage quality. University of Wisconsin Research Bull. R2786 34 pp.
- Undersander, D., N.P. Martin, D. Cosgrove, K. Kelling, M.A. Schmitt, J. Wedberg, R.L. Becker, C. Grau, J. Doll, and M.E. Rice. 2000. Alfalfa management guide. ASA, CSSA, and SSSA, Madison WI.
- Volenc, J.J., and J.H. Cherney. 1990. Yield components, morphology and forage quality of multifoliolate alfalfa phenotypes. Crop Sci. 30:1234–1238.
- Volenc, J.J., J.H. Cherney, and K.D. Johnson. 1987. Yield components, plant morphology, and forage quality of alfalfa as influenced by plant population. Crop Sci. 27:321–326.